AD_____

AWARD NUMBER DAMD17-98-1-8050

TITLE: Cytochrome P450-17alpha Polymorphism and Risk of Breast Cancer

PRINCIPAL INVESTIGATOR: Habibul Ahsan, M.D.

CONTRACTING ORGANIZATION: Columbia University
New York, New York 10032

REPORT DATE: August 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

DTIC QUALITY INSPECTED 3

20010109 101

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE August 1999	3. REPORT TYPE AND DATES COVERED Annual (1 Aug 98 - 31 Jul 99)

4. TITLE AND SUBTITLE
Cytochrome P450-17alpha Polymorphism and Risk of Breast Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8050

6. AUTHOR(S)
Habibul Ahsan, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Columbia University
New York, New York 10032

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The current study investigates whether polymorphisms in the CYP17 gene, which plays roles in estrogen biosynthesis, are associated with an altered risk of breast cancer in a population-based sample of women (400 cases and 400 controls) who participated in the Long Island Breast Cancer Study Project (LIBSCP). In addition, we are also exploring whether the effects of reproductive risk factors and exposure to exogenous estrogen are modified by CYP17 polymorphisms. In the past year, laboratory assays on CYP17 polymorphisms are being conducted in the Division of Environmental Health Sciences of Columbia University. The polymorphisms are being determined by PCR-RFLP methods with a digestion by appropriate restriction enzymes. To date, assays on 300 cases and 300 controls have been performed. Delays in subject and sample selection in the parent LIBSCP study delayed Task 1, thus delaying the start of laboratory analyses. Therefore, Task 2 will not be completed until the end of September 1999. Entry of laboratory data to computers and merging of laboratory data with questionnaire data (Task 3) are estimated to begin in October 1999. Data analysis, manuscript preparation and report writing (Tasks 4 & 5) will take place in the coming year.

14. SUBJECT TERMS
Breast Cancer, CYP17, estrogen, epidemiology

15. NUMBER OF PAGES
6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

_____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

_✓_ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

_____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

_____   8/27/99
PI - Signature   Date

Cytochrome P450-17alpha Polymorphism and Risk of Breast Cancer
PI: Habibul Ahsan, MD

Table of Contents

Introduction
Cumulative exposure of circulating estrogen to the breast is linked to the etiology of breast cancer with several studies[1,2] suggesting that circulating estradiol (the active form of estrogen) is significantly elevated in breast cancer patients compared to controls in both high and low risk populations[3-6]. This variation in circulating estradiol may be due in part to genetic polymorphisms affecting the activity of the enzymes responsible for the metabolism and cellular binding of estrogen in individuals. The enzyme, whose polymorphisms have demonstrated the most potential in the etiology of breast cancer, is the cytochrome P450c17α (CYP17) enzyme. Therefore, the current investigation is investigating whether polymorphisms of the CYP17 gene are associated with an altered risk of breast cancer in a population-based sample of women (400 controls and 400 cases) who participated in the Long Island Breast Cancer Study Project (LIBCSP). Further, we are also exploring whether the effects of reproductive risk factors (e.g., age at menarche, menopause, last child birth and parity) and exposure to exogenous estrogen like hormone replacement therapy, oral contraceptives or estrogen-mimicking substances such as organochlorine pesticides on breast cancer are modified by CYP17 polymorphisms.

Body
Subject and sample selection was delayed in the parent study (LIBSCP) and thus Task I which involved randomly selecting 400 cases and 400 controls (frequency matched to cases by five-year age groups) in batches, using code numbers from among women who had participated in the LIBCSP, was not completed until Month 4. These women provided blood samples and completed a questionnaire for the LIBSCP. Approximately 75% of study participants are postmenopausal in this randomly selected subgroup of the LIBCSP. As expected, the majority of cases and controls randomly selected for this study reflected the racial make-up of the LIBSCP population which is 90% Caucasian, 7% Black and 3% other races.

All laboratory analyses are being done in the laboratory of Dr. Regina Santella in the Division of Environmental Health Sciences of Columbia University. Genomic DNA was isolated from all cases and controls using standard phenol/chloroform extraction method. Polymorphism in the CYP17 gene is being determined by PCR-RFLP methods with the use of appropriate restriction enzymes. Briefly, genomic DNA was amplified by PCR using CYP17-specific primers. Following PCR amplification, digestion of amplified DNA was accomplished by Msp1A1 restriction enzyme. Restriction Fragment Length Polymorphism (RFLP) patterns are being examined by agarose gel electrophoresis after staining with ethidium bromide to detect CYP17 A1 and A2 alleles. The polymorphisms are being identified as follows: 1) a base change in both alleles (A2/A2 genotype) identified if two bands of DNA on the gel are generated, 2) heterozygous (A1/A2) individuals are identified as having three bands on the gel since the enzyme cuts only the mutated allele and not the wild type allele, and 3) individuals who are homzygous (A1/A1) for the alleles produce one band of the uncut PCR product on gel. CYP17 assays completed to date were performed and interpreted blinded to subject characteristics and case-control status. To date these laboratory assays have been performed on only 300 cases and 300 controls as a result of the delay in beginning Task I, as previously mentioned. However, laboratory assays for the remaining cases and controls are expected to be completed by the end of September 1999. Task 3, which includes entry of laboratory data to computers and merging of laboratory data with questionnaire data will be completed in October/November 1999.

Data analysis (Task 4) as well as manuscript preparation and report writing (Task 5) will commence in the coming year.

Key Research Accomplishments
Not applicable at this time.

Reportable Outcomes
Not applicable at this time.

Conclusions
Not applicable at this time.

References:
1. Feigelson HS, Henderson BE. Estrogens and breast cancer. Carcinogenesis (Lond) 1996; 17: 2279-84.
2. Hulka BS, Liu ET, Lininger RA. Steroid hormones and risk of breast cancer. Cancer 1994: 74(Suppl): 1111-24.
3. Bernstein L, Yuan JM, Ross RK, et al. Serum hormone levels in premenopausal Chinese women in Shanghai and white women in Los Angeles: results from two breast cancer case-control studies. Cancer Causes Control 1990; 1:51-8.
4. Henderson BE, Gerkins V, Rosario I, Casagrande JT, Pike MC. Elevated serum levels of estrogen and prolactin in daughters of patients with breast cancer. New England J Med 1975;293:790-5.
5. Shimizu H, Ross RK, Bernstein L, Pike MC, Henderson BE. Serum estrogen levels in postmenopausal women: comparison of American whites and Japanese in Japan. Br J Cancer 1990; 62: 451-3.
6. England P, Skinner L, Cottrell K, Sellwood RA. Serum estradiol 17β in women with benign and malignant breast disease. Br J Cancer 1974; 300:571-6.